White Paper on Species/Strain/Stock in Endocrine Assays

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White Paper on Species/Strain/Stock in Endocrine Assays

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For:

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Reviewed but not in complete concurrence:

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Background

- There is evidence that different species and strains within species exhibit differing sensitivities to endocrine-active compounds
- Selection of appropriate species and strain(s), or at least understanding their differential responsivity, is important in EDSP assays
- EPA testing guidelines recommend using the rat but not strains with low fecundity. The most commonly used rat strain for these guideline studies is the Sprague-Dawley rat
- In the December 2001 meeting of the EDMVS, committee members discussed strains and stocks and concluded that the EPA should prepare a white paper summarizing what is known about interspecies and intraspecies strain/stock similarities and differences in responses to EACs, and provide the rationale for strain/stock selection

Concern

Animal models used in assays to detect endocrine disruption have been chosen on the basis of convenience and familiarity, and species/strains/stocks which are more frequently used are those which are bred specifically for robust fecundity and likely reduced sensitivity to endocrine perturbations (NTP's Report of the Endocrine Disruptors Low Dose Peer Review, 2000).

Purpose

To summarize the interspecies and intraspecies similarities and differences in response to endocrine endpoints, in order to determine whether specific species/strains should be preferred or avoided when screening for endocrine activity.

Literature Search Strategy

- Databases searched included MedLine, PubMed, Biological Abstracts, Chemical Abstracts, Toxline including DART (Developmental and Reproductive Toxicology) for published articles/abstracts
- For intraspecies comparisons, the focus was on “rat strain.” When there was a paucity of references pertaining to a general endocrine endpoint, “mouse strain” was added to the search. For interspecies comparisons, the focus was on rats and mice.
- Search Terms: “rat strain” and keywords from EDSP protocols, in addition to specific strains and specific authors

Scope

- Endocrine endpoints in assays under consideration by EDSP
- Intraspecies and interspecies studies conducted in a single laboratory (to minimize confounders); since these studies were few, multiple laboratory comparisons were also used when necessary and/or appropriate.
- Published data
- Focus on rat strains

Inbred Versus Outbred Strains

Inbred

- > 20 generations of inbreeding
- Known genetic background
- Less variable response to EACs
- Small litter size
- Less historical data

Outbred

- < 1% inbreeding/generation
- Variable genetic background
- More diverse responses to EACs
- Large litter size (due to selection for high fecundity)
- More historical data

Confounders Affecting Comparisons of Reproductive Toxicity Data

Same laboratory, different times or different laboratories.

- **Animals**
  - ***Source/supplier*** (the same strain from different suppliers will most likely be genetically different)
  - ***Age, weight, and health status***
- **Husbandry**
  - ***Housing***
  - ***Caging/water bottles***
  - ***Feed and Water***
  - ***Temperature and relative humidity and Light cycle***
  - ***Technician skills and experience***
  - ***Source of the test material***
- **Study Design**
  - ***Number of animals/dose groups, dose levels, vehicle, route***
  - ***Data (how collected and analyzed)***

Endocrine Endpoints in EDSP Assays

- Fertility and Gestational Indices
- Survival and Growth Indices
- Reproductive Tract Development
- Urethral Vaginal Distance (UVD)
- Vaginal Patency in Females
- Age of First Estrus in Females
- Estrous Cyclicity
- Uterine Weight
- Anogenital Distance (AGD)
- Retention of Nipples/Areolae in Preweanling Males
- Preputial Separation in Males
- Sex Accessory Structures
- Andrology
- Behavioral Assessments (Clinical Observations)
- Hormonal Controls
- Gross Examinations
- Organ Weights and Histopathology

Summary of Agent- and Endpoint-Specific Intraspecies Differences

Endocrine Endpoint	Chemical	Sensitive Strains	Less Sensitive/ Insensitive Strains	References (from Table 2 of the white paper)
Uterine Weight	EE	Wistar, Da/Han	SD	1
	BPA	Da/Han	Wistar, SD	1
	NP	AP>SD		2
	EE, DES	SD, F344		3
	D4	SD	F344	4
	E2	SD,F344		6
	tamoxifen	SD	F344	6
AGD	p,p'-DDE	LE	SD	7
	flutamide	SD, LE		7
Nipple retention	p,p'-DDE	SD	LE	7
	flutamide	SD, LE		7
	vinclozolin	LE > Wistar		14

Summary of Agent- and Endpoint-Specific Intraspecies Differences (Continued)

Endocrine Endpoint	Chemical	Sensitive* Strains	Less Sensitive/ Insensitive Strains	References (from Table 2 of the white paper)
PPS	E2	F344, SD		8
	p,p'-DDE		SD, LE	7
VO	p,p'-DDE		SD, LE	7
	BPA	AP	SD	9
Male reproductive organ wts.	flutamide	LE, SD		7
	E2	F344, SD		8
	low dose E2	SD	F344	8
	vinclozolin	LE	Wistar	14
	BPA		C57BL/6N, ICR mouse	38
	E2	C57BL/6N mouse		38
	E2	B6, C17/Jls mouse	ICR, CD-1, S15 mouse	39
	DEHP	CD-1 mouse	Jcl:ICR mouse	37

Summary of Agent- and Endpoint-Specific Intraspecies Differences (Continued)

Endocrine Endpoint	Chemical	Sensitive* Strains	Less Sensitive/ Insensitive Strains	References (from Table 2 of the white paper)
Estrous cycle/ovulation	feed restriction	F344, BN	SD, LE	18
	atrazine	LE	SD	21
	atrazine	SD	F344	22
Fertility/gestational effects	atrazine	Holtzman	F344, SD, LE	29
	atrazine	F344	SD, LE	30
	BDCM	F344	SD	31
Andrology	BPA	AP	SD	15
	lead	SD		17
	E2	B6, C17/Jls mouse	CD-1, S15 mouse	39
Hormone Levels	p,p'DDE	SD (*FSH, E2, T4*)	LE (*FSH, Prl, LH*)	13

Summary of Agent- and Endpoint-Specific Intraspecies Differences (Continued)

Endocrine Endpoint	Chemical	Sensitive* Strains	Less Sensitive/ Insensitive Strains	References (from Table 2 of the white paper)
Hormone Levels	p,p'DDE	SD (*FSH, E2, T4*)	LE (*FSH, Prl, LH*)	13
	p,p'DDE	LE (*E2, T4, T, DHT, TSH*)	SD (*Prl, LH, T, DHT, TSH*)	13
	E2	SD (*Prl*)	F344 (*P*rl)	22
	TCDD	Han/Wistar (*T, LH*)	LE (*T, LH*)	23
	atrazine	LE (*LH, Prl*)	SD (*LH, Prl*)	24
	atrazine	Holtzman (*P*)	SD (E2, *P*)	26
	E2	F344 (*Prl*)	SD *(Prl*)	25
	BPA	F344 (*Prl*)	SD (*Prl*)	25
	TCDD	LE (*T4*)		27
	TSH, TRH	SD, F344 (*T4*)	SD (*T3*)	28
	TSH, TRH	F344 (*T3*)		28

Summary of Agent- and Endpoint-Specific Intraspecies Differences (Continued)

Endocrine Endpoint	Chemical	Sensitive* Strains	Less Sensitive/ Insensitive Strains	References (from Table 2 of the white paper)
Pituitary Weights	E2	F344	SD	33
	E2	F344>BN	Wistar, Donryu	34
	DES	F344	SD, BN	35
Histopathology (reproductive organs)	BPA	F344 (females)	SD (females)	10
	DMAB	F344>ACI>Lewis>CD (males)	Wistar (males)	12
	vinclozolin	LE (males)	Wistar (males)	14
	cadmium	F344 (females)	WF (cadmium)	40
	atrazine	SD (females)	F344 (females)	22

References for Summary Tables

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Rat Interstrain Comparisons
(based on current data)

Outbred

- Uterine weight affected by many chemicals
- AGD and nipple retention, sensitive in some (depending on chemical)
- Male reproductive organs affected by variety of chemicals
- Effects on hormone levels are dependent on the hormone measured and chemical

Inbred

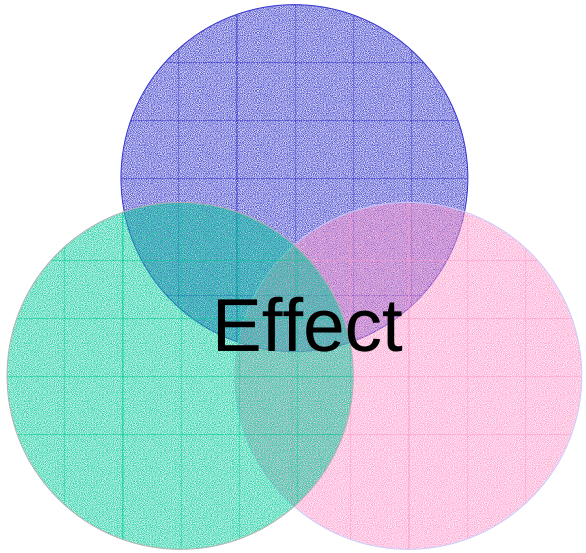
- Greater effects of chemicals on pituitary weight
- Uterine weight less affected
- More sensitive to gestation/fertility effects
- Effects on hormone levels are dependent on the hormone measured and chemical
- Comparisons based mostly on F344 strain (little data in other inbred strains)

Interspecies Similarities and Differences

- Few studies have been conducted in a single laboratory comparing the effects of endocrine-disrupting chemicals in more than one strain within a species, and even fewer studies have been conducted in a single laboratory comparing the effects of endocrine-disrupting chemicals in more than one species.
- Difficult to compare species when variability across strains within a species is high
- NTP studies of the effects of 24 different chemicals on male reproductive parameters in B6 mice and F344 rats across 7 labs show a 58% correlation in response to reproductive toxicants (even with the same rat and mouse strains).

Genetic Differences in Response to Endocrine-Active Chemicals

Chemical



Endpoint

Genotype

There are strain (genotype) by environmental agent by endpoint interactions. These need to be considered in selecting the appropriate species/strains for EDSP assays.

Conclusions

- Comparisons revealed variability in effects produced by endocrine-disrupting chemicals on endocrine endpoints from strain to strain. Endocrine effects were chemical specific, strain specific, endpoint specific, and, in some cases, laboratory specific. There were more sensitive and less sensitive strains to endocrine-active compounds among both outbred and inbred strains, depending on the chemical used and the endpoints evaluated.
- Inbred strains are homogeneous at all loci, and have a limited range of responses (less variability, but an effect may be missed), so using several genetically-defined inbred strains in endocrine screens may be the only way to provide a broad spectrum of responsivity. If selecting a single strain for endocrine screens, outbred strains have more genetic variability, exhibit a broader range of responsivity (with a greater likelihood of detecting an effect), and may be more appropriate. Outbred strains, which are heterogeneous like humans and other species of interest, may provide a more appropriate animal model for determining the effects of EACs.

Conclusions (continued)

- Since the actions of EACs were generally observed for more than one endpoint, there is a greater likelihood of detecting an endocrine disruptor in a study with many endpoints.
- In current OECD and EPA validation efforts for the Uterotrophic and Hershberger Assays (looking at many of the same endpoints), there was no effect on responsivity of different strains (housing, feed, bedding, etc.) with potent androgens and estrogens.

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